AMENDMENTS TO THE CLAIMS

Claims 1-7 (Cancelled)

Claim 8 (Currently amended): A method of isolating a population of rAAV particles, comprising the steps of:

(a) chromatographing an AAV producer cell lysate containing rAAV particles on a positively-charged anion exchange resin; ~~and (b)~~ or chromatographing an AAV producer cell lysate containing rAAV particles on a negatively-charged cation exchange resin, and collecting a fraction containing rAAV particles; and

(b) chromatographing the fraction of step (a) on exchange resin opposite in charge to that used in step (a) and collecting a fraction containing rAAV particles;

whereby a purified population of rAAV particles is generated.

Claim 9 (Currently amended): The method of claim 8, wherein ~~step (a) is performed before step (b)~~ chromatographing on a positively-charged anion exchange resin is performed before chromatographing on a negatively-charged cation exchange resin.

Claim 10 (Currently amended): The method of claim 8, wherein ~~step (b) is performed before step (a)~~ chromatographing on a negatively-charged cation exchange resin is performed before chromatographing on a positively-charged anion exchange resin.

Claim 11 (Currently amended): The method of claim 10, further comprising step (c) of chromatographing the ~~lysate~~ fraction containing rAAV particles from step (b) on a negatively-charged cation exchange resin~~, said step performed after steps (a) and (b)~~.

Claim 12 (Original): The method of claim 11, wherein heparin sulfate is used for step (c).

Claim 13 (Original): The method of claim 8, further comprising the step of subjecting the producer cells to tangential flow filtration.

Claim 14 (Currently amended): The method of claim 8, wherein the lysate is subjected to tangential flow filtration prior to step (a).

Claim 15 (Cancelled)

Claim 16 (Currently amended): The method of claim [[14]] 8, wherein the fraction containing rAAV particles from step (a) or step (b) is subject to tangential flow filtration ~~is performed after chromatography~~.

Claim 17 (Original): The method of claim 8, wherein said anion exchange resin is an N-charged amino or imino resin.

Claim 18 (Currently amended): The method of claim 17, wherein said anion exchange resin is selected from the group consisting of ~~a POROS 50 PI resin,~~ a diethylaminoethyl (DEAE) resin, a trimethylaminoethyl (TMAE) resin, a quaternary amine resin and a ~~polyethylenimine~~ polyethyleneimine (PEI) resin.

Claim 19 (Original): The method of claim 8, wherein said cation exchange resin is a sulfo-, phospho- or carboxy-based cationic resin.

Claim 20 (Currently amended): The method of claim 19, wherein said cation exchange resin is selected from the group consisting of [[an]] a heparin sulfate (HS) resin, [[an]] a sulphopropyl (SP) resin, and a carboxymethyl (CM) resin.

Claim 21 (Original): The method of claim 8, wherein the producer cell is cultured under suspension conditions.

Claim 22 (Currently amended): A method of isolating a population of rAAV particles, comprising the steps of:

(a) chromatographing an AAV producer cell culture supernatant which contains rAAV particles on a positively-charged anion exchange resin~~; and (b)~~ or chromatographing [[the]] an AAV producer cell culture supernatant containing rAAV particles on a negatively-charged cation exchange resin, and collecting a fraction containing rAAV particles; and

(b) chromatographing the fraction of step (a) on exchange resin opposite in charge to that used in step (a) and collecting a fraction containing rAAV particles;

whereby a purified population of rAAV particles is generated.

Claim 23 (Currently amended): The method of claim 22, wherein ~~step (a) is performed before step (b)~~ chromatographing on a positively-charged anion exchange resin is performed before chromatographing on a negatively-charged cation exchange resin.

Claim 24 (Currently amended): The method of claim 22, wherein ~~step (b) is performed before step (a)~~ chromatographing on a negatively-charged cation exchange resin is performed before chromatographing on a positively-charged anion exchange resin.

Claim 25 (Currently amended): The method of claim 24, further comprising step (c) of chromatographing the ~~lysate~~ fraction containing rAAV particles from step (b) on a negatively-charged cation exchange resin~~, said step performed after steps (a) and (b)~~.

Claim 26 (Original): The method of claim 25 wherein heparin sulfate is used for step (c).

Claim 27 (Currently amended): The method of claim 22, further comprising the step of subjecting the culture supernatant to tangential flow filtration prior to step (a).

Claim 28 (Cancelled)

Claim 29 (Currently amended): The method of claim [[27]] 22, wherein the faction containing rAAV particles from step (a) or step (b) is subject to tangential flow filtration ~~is performed after chromatography~~.

Claim 30 (Original): The method of claim 22, wherein said anion exchange resin is an N-charged amino or imino resin.

Claim 31 (Currently amended): The method of claim 30, wherein said anion exchange resin is selected from the group consisting of ~~a POROS 50 PI resin,~~ a diethylaminoethyl (DEAE) resin, a trimethylaminoethyl (TMAE) resin, a quaternary amine resin and a ~~polyethylenimine~~ polyethyleneimine (PEI) resin.

Claim 32 (Original): The method of claim 22, wherein said cation exchange resin is a sulfo-, phospho- or carboxy-based cationic resin.

Claim 33 (Currently amended): The method of claim 32, wherein said cation exchange resin is selected from the group consisting of [[an]] a heparin sulfate (HS) resin, [[an]] a sulphopropyl (SP) resin, and a carboxymethyl (CM) resin.

Claim 34 (Original): The method of claim 22, wherein the producer cell is cultured under suspension conditions.

Claims 35-49 (cancelled)

Claim 50 (Previously Presented): A method of generating a population of recombinant adeno-associated virus (rAAV) particles, comprising the steps of:

a) incubating an AAV producer cell under conditions that are permissive for replication of AAV; said producer cell comprising: (i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein; (ii) a recombinant AAV (rAAV) vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and (iii) a helper virus for AAV;

b) lysing the producer cell after the incubation of step a) to produce an AAV producer cell lysate;

c) chromatographing the AAV producer cell lysate of step b) on at least one positively-charged anion exchange resin; and

d) purifying the chromatographic fractions containing rAAV particles of step c) by cation exchange chromatography or tangential flow filtration to generate a purified population of rAAV vector particles.

Claim 51 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein said purifying step d) comprises subjecting the fractions to cation exchange chromatography.

Claim 52 (Cancelled)

Claim 53 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein said rAAV vector comprises a heterologous non-AAV polynucleotide flanked by two AAV inverted terminal repeats (ITRs).

Claim 54 (Original): A method of generating a population of rAAV particles according to claim 50, wherein said AAV producer cell comprises at least one AAV packaging gene that is stably integrated into the genome of said AAV producer cell.

Claim 55 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein the helper virus is introduced into the producer cell already introduced with the AAV packaging gene(s) and the rAAV vector.

Claim 56 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein the rAAV vector and the helper virus are introduced simultaneously or sequentially into the producer cell already introduced with the AAV packaging gene(s).

Claim 57 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein the AAV packaging gene(s) and the rAAV vector are introduced simultaneously or sequentially into the producer cell already introduced with the helper virus.

Claim 58 (Original): A method of generating a population of rAAV particles according to claim 50, wherein said AAV producer cell comprises an AAV *rep* gene and an AAV *cap* gene.

Claim 59 (Original): A method of generating a population of rAAV particles according to claim 50, wherein said AAV *rep* gene and AAV *cap* gene are stably integrated into the genome of said AAV producer cell.

Claim 60 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein at least one AAV split-packaging gene is introduced into the producer cell.

Claim 61 (Original): A method of generating a population of rAAV particles according to claim 50, wherein said helper virus is an adenovirus.

Claim 62 (Original): A method of generating a population of rAAV particles according to claim 50, wherein said helper virus is a temperature-sensitive helper virus and said step of incubating the producer cell is conducted at a temperature that is permissive for replication of AAV but non-permissive for replication of the temperature-sensitive helper virus.

Claim 63 (Original): A method of generating a population of rAAV particles according to claim 50, wherein said helper virus is a temperature-sensitive adenovirus.

Claim 64 (Original): A method of generating a population of rAAV particles according to claim 50, wherein said helper virus is adenovirus Ad-ts149.

Claim 65 (Original): A method of generating a population of rAAV particles according to claim 50, wherein said AAV producer cell lysate is also affinity purified on a resin having a ligand that is specific for one or more surface molecules present on AAV.

Claim 66 (Original): A method of generating a population of rAAV particles according to claim 65, wherein the affinity purification is conducted after ion-exchange chromatography.

Claim 67 (Original): A method of generating a population of rAAV particles according to claim 65, wherein said ligand is an antibody that is specific for a surface molecule present on AAV.

Claim 68 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cells of step a) are concentrated prior to lysis.

Claim 69 (Previously Presented): A method of generating a population of rAAV particles according to claim 68, wherein the AAV producer cells of step a) are concentrated by centrifugation or by tangential flow filtration prior to lysis.

Claim 70 (Original): A method of generating a population of rAAV particles according to claim 50, wherein said step of lysing the AAV producer cell is conducted by subjecting the cells to a lytic technique selected from the group consisting of microfluidization, sonication, and freeze-thawing.

Claim 71 (Original): A method of generating a population of rAAV particles according to claim 70, wherein said step of lysing the AAV producer cell is conducted by subjecting the cells to microfluidization.

Claim 72 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cell lysate of step b) is treated with a nuclease prior to chromatography.

Claim 73 (Original): A method of generating a population of rAAV particles according to claim 72, wherein said nuclease is Benzonase.

Claim 74 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cell lysate of step b) is clarified prior to chromatography.

Claim 75 (Previously Presented): A method of generating a population of rAAV particles according to claim 74, wherein the AAV producer cell lysate of step b) is clarified by filtration or centrifugation prior to chromatography.

Claim 76 (Original): A method of generating a population of rAAV particles according to claim 50, wherein the AAV producer cells are concentrated prior to lysis, resuspended in a buffer comprising saline at an ionic strength at least that of a 50 mM NaCl solution, lysed, and then clarified by filtration prior to chromatography.

Claim 77 (Original): A method of generating a population of rAAV particles according to claim 51, wherein chromatographic fractions containing rAAV particles are concentrated by filtration or centrifugation after elution from the chromatographic resin.

Claim 78 (Original): A method of generating a population of rAAV particles according to claim 51, wherein chromatographic fractions containing rAAV particles are concentrated by tangential flow filtration.

Claim 79 (Original): A method of generating a population of rAAV particles according to claim 50, wherein said anion exchange resin is an N-charged amino or imino resin.

Claim 80 (Currently amended): A method of generating a population of rAAV particles according to claim 50, wherein said anion exchange resin is selected from the group consisting of ~~a POROS 50 PI resin,~~ a diethylaminoethyl (DEAE) resin, a trimethylaminoethyl (TMAE) resin, a quaternary amine resin and a ~~polyethylenimine~~ polyethyleneimine (PEI) resin.

Claim 81 (Original): A method of generating a population of rAAV particles according to claim 51, wherein said cation exchange resin is a sulfo-, phospho- or carboxy-based cationic resin.

Claim 82 (Previously Presented): A method of generating a population of rAAV particles according to claim 51, wherein said cation exchange resin is selected from the group consisting of a heparin sulfate (HS) resin, a sulfopropyl (SP) resin, and a carboxymethyl (CM) resin.

Claim 83 (Original): A method of generating a population of rAAV particles according to claim 50, wherein the producer cell of step a) is an attachment-dependent mammalian cell line.

Claim 84 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein said step a) of incubating the producer cell is conducted in a vessel selected from the group consisting of a tissue culture flask, a roller bottle, a spinner flask, a tank reactor, a fermentor, and a bioreactor.

Claim 85 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein said step a) of incubating the producer cell is conducted using a microcarrier.

Claim 86 (Previously Presented): A method of generating a population of rAAV particles according to claim 84, wherein said bioreactor is a hollow-fiber, packed-bed or fluidized-bed bioreactor.

Claim 87 (Original): A method of generating a population of rAAV particles according to claim 50, wherein the producer cell of step a) is a suspension-adapted mammalian cell line.

Claim 88 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein said step a) of incubating the producer cell is conducted in a vessel selected from the group consisting of a spinner flask, a tank reactor and an air lift fermentor.

Claim 89 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein said step a) of incubating the producer cell is performed in rAAV medium essentially as shown in Table 2.

Claim 90 (Original): A method of generating a population of rAAV particles according to claim 50, wherein the producer cells are 293 N3s cells or HeLa S3 cells.

Claim 91 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein step a) is conducted for at least 5 days.

Claim 92 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein step a) of incubating the producer cell is conducted in a multi-liter bioreactor and wherein at least about 10^9 replicative units of rAAV per liter of bioreactor volume are isolated after step d).

Claims 93-117 (Cancelled)

Claim 118 (Previously presented): A method of generating a population of recombinant adeno-associated virus (rAAV) particles, comprising the steps of:

a) incubating an AAV producer cell under conditions that are permissive for replication of AAV and which comprise inducing a stress in the AAV producer cell; wherein said AAV producer cell comprising (i) one or more AAV packaging genes, wherein each said AAV packaging gene encodes an AAV replication or encapsidation protein; (ii) a recombinant AAV (rAAV) vector that comprises a heterologous non-AAV polynucleotide flanked by at least one AAV inverted terminal repeat (ITR); and (iii) a helper virus for AAV;

b) lysing the producer cell after the incubation of step a) to produce an AAV producer cell lysate; and

c) purifying the AAV producer cell lysate to generate a population of recombinant adeno-associated virus (rAAV) particles, wherein said purifying step comprises chromatographing the AAV producer cell lysate of step b) on at least one positively-charged anion exchange resin followed by purifying on either a cation exchange resin or by tangential flow filtration to generate a purified population of rAAV vector particles.

Claim 119 (Previously Presented): The method of claim 118, wherein said purifying step c) comprises chromatographing the AAV producer cell lysate of step b) on at least one negatively-charged cation exchange resin followed by purifying on an anion exchange resin.

Claims 120-177 (Cancelled)

Claim 178 (Previously Presented): A method of generating a population of rAAV particles according to claim 50, wherein said purifying step d) comprises subjecting the fractions to tangential flow filtration.

Claim 179 (Previously Presented): A method of generating a population of rAAV particles according to claim 178, wherein the tangential flow filtration is performed with diafiltration using a solution comprising 5% glycerol.

Claim 180 (Previously Presented): The method of claim 118, wherein said purifying step c) comprises chromatographing the AAV producer cell lysate of step b) on at least one positively-charged anion exchange resin followed by purifying on a cation exchange resin.

Claim 181 (Previously Presented): The method of claim 118, wherein said purifying step c) comprises chromatographing the AAV producer cell lysate of step b) on at least one positively-charged anion exchange resin followed by purifying on a tangential flow filtration.

Claim 182 (Previously Presented): The method of claim 181, wherein the tangential flow filtration is performed with diafiltration using a solution comprising 5% glycerol.

Claims 183 - 184 (Cancelled)

Claim 185 (Previously Presented): A method of isolating rAAV particles comprising the steps of:

(a) chromatographing an AAV producer cell lysate containing the rAAV particles on a positively charged anion exchange resin; and

(b) purifying the chromatographic fractions containing the rAAV particles of step a) by tangential flow filtration to generate a purified population of the rAAV particles.

Claim 186 (Previously Presented): The method of claim 185, wherein said anion exchange resin is an N-charged amino or imino resin.

Claim 187 (Currently amended): The method of claim 185, wherein said anion exchange resin is selected from the group consisting of ~~a POROS 50 PI resin,~~ a diethylaminoethyl (DEAE) resin, a trimethylaminoethyl (TMAE) resin, a quaternary amine resin and a ~~polyethylenimine~~ polyethyleneimine (PEI) resin.

Claim 188 (Previously Presented): The method of claim 185, wherein the producer cell is cultured under suspension conditions.

Claim 189 (Previously Presented): The method of claim 185, wherein the tangential flow filtration is performed with diafiltration using a solution comprising 5% glycerol.

Claim 190 (Previously Presented): A method of isolating rAAV particles comprising the steps of:

(a) chromatographing an AAV producer cell culture supernatant which contains the rAAV particles on a positively charged anion exchange resin; and

(b) purifying the chromatographic fractions containing the rAAV particles of step a) by tangential flow filtration to generate a purified population of the rAAV particles.

Claim 191 (Previously Presented): The method of claim 190, wherein said anion exchange resin is an N-charged amino or imino resin.

Claim 192 (Currently amended): The method of claim 190, wherein said anion exchange resin is selected from the group consisting of ~~a POROS 50 PI resin,~~ a diethylaminoethyl (DEAE) resin, a trimethylaminoethyl (TMAE) resin, a quaternary amine resin and a polyethylenimine (PEI) resin.

Claim 193 (Previously Presented): The method of claim 190, wherein the producer cell is cultured under suspension conditions.

Claim 194 (Previously Presented): The method of claim 190, wherein the tangential flow filtration is performed with diafiltration using a solution comprising 5% glycerol.

Claim 195 (Previously Presented): The method of claim 119, wherein the method further comprises a step of purifying the chromatographic fractions containing rAAV particles of step c) by cation exchange resin.

Claim 196 (New): The method of claim 19, wherein said cation exchange resin is a sulfo-based cationic resin.

Claim 197 (New): The method of claim 19, wherein said cation exchange resin is a phospho-based cationic resin.

Claim 198 (New): The method of claim 19, wherein said cation exchange resin is a carboxy-based cationic resin.

Claim 199 (New): The method of claim 9, wherein the anion exchange resin is a polyethyleneimine (PEI) resin and the cation exchange resin is a sulphopropyl (SP) resin.

Claim 200 (New): The method of claim 10, wherein the cation exchange resin is a sulphopropyl (SP) resin and the anion exchange resin is a polyethyleneimine (PEI) resin.

Claim 201 (New): The method of claim 200, further comprising step (c) of chromatographying the fraction containing rAAV particles from step (b) on a heparin sulfate resin.

Claim 202 (New): The method of claim 32, wherein said cation exchange resin is a sulfo-based cationic resin.

Claim 203 (New): The method of claim 32, wherein said cation exchange resin is a phospho-based cationic resin.

Claim 204 (New): The method of claim 32, wherein said cation exchange resin is a carboxy-based cationic resin.

Claim 205 (New): The method of claim 23, wherein the anion exchange resin is a polyethyleneimine (PEI) resin and the cation exchange resin is a sulphopropyl (SP) resin.

Claim 206 (New): The method of claim 24, wherein the cation exchange resin is a sulphopropyl (SP) resin and the anion exchange resin is a polyethyleneimine (PEI) resin.

Claim 207 (New): The method of claim 206, further comprising step (c) of chromatographying the fraction containing rAAV particles from step (b) on a heparin sulfate resin.